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Generation of transgenic porcine chimeras using primordial germ cell-derived colonies.

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In mice, two pluripotent cell lines, embryonic stem (ES) cells and embryonic germ (EG) cells, have been identified. We present here results indicating that porcine EG cell lines can be isolated, genetically transformed, and utilized to make transgenic chimeras. Briefly, primordial germ cells (PGCs) were isolated from Day 25-27 fetuses and plated on STO feeder cells in Dulbecco's modified Eagle's medium:Ham's F-10 medium supplemented with 0.01 mM nonessential amino acids, 2 mM glutamine, 15% fetal bovine serum, 0.1 mM 2-mercaptoethanol, 40 ng/ml human stem cell factor, 20 ng/ml human basic fibroblast growth factor, and 20 ng/ml human leukemia inhibitory factor. For genetic transformation, cells were electroporated with a construct containing the green fluorescent protein under control of the cytomegalovirus promoter. After electroporation, cells were plated and later examined under fluorescein isothiocyanate excitation. Fluorescent colonies were selected for chimera generation. Blastocysts collected from gilts on Day 5 were injected with 10-15 transgenic PGC-derived cells and transferred into recipient gilts. Gilts were hysterectomized on Day 25, and fetal tissues were analyzed by Southern blotting. Three chimeras out of 20 fetuses analyzed were transgenic. Additionally, when one recipient gilt was allowed to go to term, one piglet with transgenic contribution was identified.